

# An ultrastructural study of the relationship between the mite *Floracarus perrepae* Knihinicki & Boczek (Acariformes: Eriophyidae) and the fern *Lygodium microphyllum* (Lygodiaceae)

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## Abstract

The ultrastructure of the mite *Floracarus perrepae* was investigated in relation to its host, *Lygodium microphyllum*, the Old World climbing fern. *Floracarus perrepae* has been suggested as a means of biological control for the fern, which is an aggressive weed in tropical areas. Feeding by the mite induces a change in the size of epidermal cells, and cell division is stimulated by mite feeding, causing the leaf margin to curl over into a roll with two to three windings. The enlarged epidermal layer greatly increases its cytoplasmic contents, which become a nutritive tissue for the mite and its progeny. Damage by the mite ultimately debilitates the fern. The structure and depth of stylet penetration by the mite, and the thickness of the epidermal cell wall of *L. microphyllum*, do not appear to account for the mite's differential ability to induce leaf rolling in its co-adapted host from south-east Queensland but not in the invasive genotype of the fern in Florida.

## Key words

biological control, electron microscopy, Florida everglades, host range, invasive species, mite–plant interactions.

## INTRODUCTION

*Floracarus perrepae* Knihinicki & Boczek (Acariformes: Eriophyidae) is a herbivore associated with *Lygodium microphyllum* (Lygodiaceae), the Old World climbing fern (Knihinicki & Boczek 2002; Goolsby *et al.* 2003). *Lygodium microphyllum* is native to the Old World wet tropics and subtropics, including Africa, Australasia, Asia and Oceania (Pemberton 1998). *Lygodium microphyllum* is an aggressive invasive weed of moist habitats in southern Florida (Pemberton & Ferriter 1998) and the target of a biological control program (Pemberton *et al.* 2002; Goolsby *et al.* 2003). *Floracarus perrepae* is one of the herbivores under consideration as a biological control agent. Several distinct genotypes of *F. perrepae* have been identified using a nuclear rRNA *D2* and mitochondrial *COI* genes (Goolsby *et al.* 2003, 2004). Genotypes of *L. microphyllum* also were characterised, using two separate chloroplast intro genes, *TrnF-TrnL* and *rps4-TrnS* (Goolsby *et al.* 2003, 2004). The mite genotype from south-

eastern Queensland performed poorly on the invasive genotype of *L. microphyllum* from Florida. The mechanism for the mite–fern interaction is not known. However, the ability of the mite genotype to feed and induce gall tissue and leaf rolling in the fern is critical to its ability to survive and reproduce. Mature females prefer the new sterile subpinnae of actively growing plant tips for oviposition. Observations of newly formed subpinnae revealed up to five adults inside, although a single gravid female is able to cause the development of the deformation (curled subpinna) for itself and all her progeny. As the subpinna margin continues to roll, the number of mites may increase significantly so that subpinnae leaf rolls ultimately may contain 30 or more adults, juveniles and eggs (SK Ozman & JA Goolsby unpubl. data 2002).

Eriophyoid mites are small with body lengths ranging from 80 to 500 µm and an average length of approximately 200 µm. These mites are highly specific, living and reproducing only on susceptible host-plant species (Westphal & Manson 1996). Mouth parts of eriophyoid mites are complex and are uniquely adapted for obligate phytophagous feeding associated with host plants (Nuzzaci 1979a; Thomsen 1987; Lindquist & Oldfield 1996). The eriophyoid mite gnathosoma includes short pedipalps, a prominent subcapitulum (rostrum) and the stylets. Depending on interpretation, stylet number ranges

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from seven to nine (Nuzzaci 1979b; Lindquist 1996). Both Lindquist (1996) and Nuzzaci and Alberti (1996) have identified nine stylets including the unpaired oral stylet, the inner infracapitular (auxiliary) stylets, the outer infracapitular stylets, and the cheliceral stylets, which divide into fixed and movable digits. The oral stylet is only about half the length of the cheliceral stylets. Movement of the cheliceral stylets, limited to an alternate back-and-forth boring motion activated by the motivator, may continue throughout the feeding process (Lindquist & Oldfield 1996).

According to Thomsen (1988), enzymatic digestion of the epidermal wall precedes food ingestion. Westphal and Manson (1996) reported that it is unlikely that salivary deposition on the host surface is involved in enzymatic dissolution of the cell walls prior to insertion of the cheliceral stylets. Lindquist (1996) and Nuzzaci and Alberti (1996) have provided an excellent review of the literature describing the structure and function of eriophyoid mites.

Penetration by the mouthparts is generally restricted to the epidermal layer of the host plant (Oldfield 1996) and feeding activity results in minimal physical and physiological damage according to Lindquist and Oldfield (1996). The cheliceral stylets of gall mites were found to penetrate only about 2 µm and affected only the host cell wall (Westphal & Manson 1996). Prior to penetration, the pedipalps are positioned against the host-plant surface with the terminal palpal segments anchoring the gnathosoma (Nuzzaci & Alberti 1996).

Our study was designed to describe both the morphology of *F. perrepae*, especially the mouthparts, and the ultrastructural changes in *L. microphyllum* subpinnae (smallest leaf units) as a result of mite feeding. Detailed knowledge of the morphology of the mouthparts of *F. perrepae* is essential to the understanding of its feeding behaviour.

## MATERIALS AND METHODS

The *F. perrepae* used in the study were collected from *L. microphyllum* at Carbrook Creek, Logan, Queensland and Scrub Hen Creek, Lockhart River, Queensland, by John A Goolsby. Infested subpinnae with tightly rolled margins were removed from the compound pinnae and prepared for light and electron microscopy. Some subpinnae were fixed and dehydrated in acidified DMP (2,2-dimethoxypropane). Other infested subpinnae were dropped into boiling water for 30 s, then fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.4). Following fixation, both samples were dehydrated in a graded ethanol series and then critical-point dried using liquid carbon dioxide as a transitional fluid. Specimens were mounted on aluminium stubs using double sticky carbon tape, sputter coated with gold or gold-palladium, and examined using a JEOL JSM 6400 or 6300 scanning electron microscope. Infested pinnae were prepared for light and transmission electron microscopy by fixation in 2.5% phosphate-buffered glutaraldehyde (pH 7.4), post-fixed in buffered osmium tetroxide (pH 7.4), dehydrated in a graded acetone series, embedded in epon-araldite epoxy resin and ultrathin

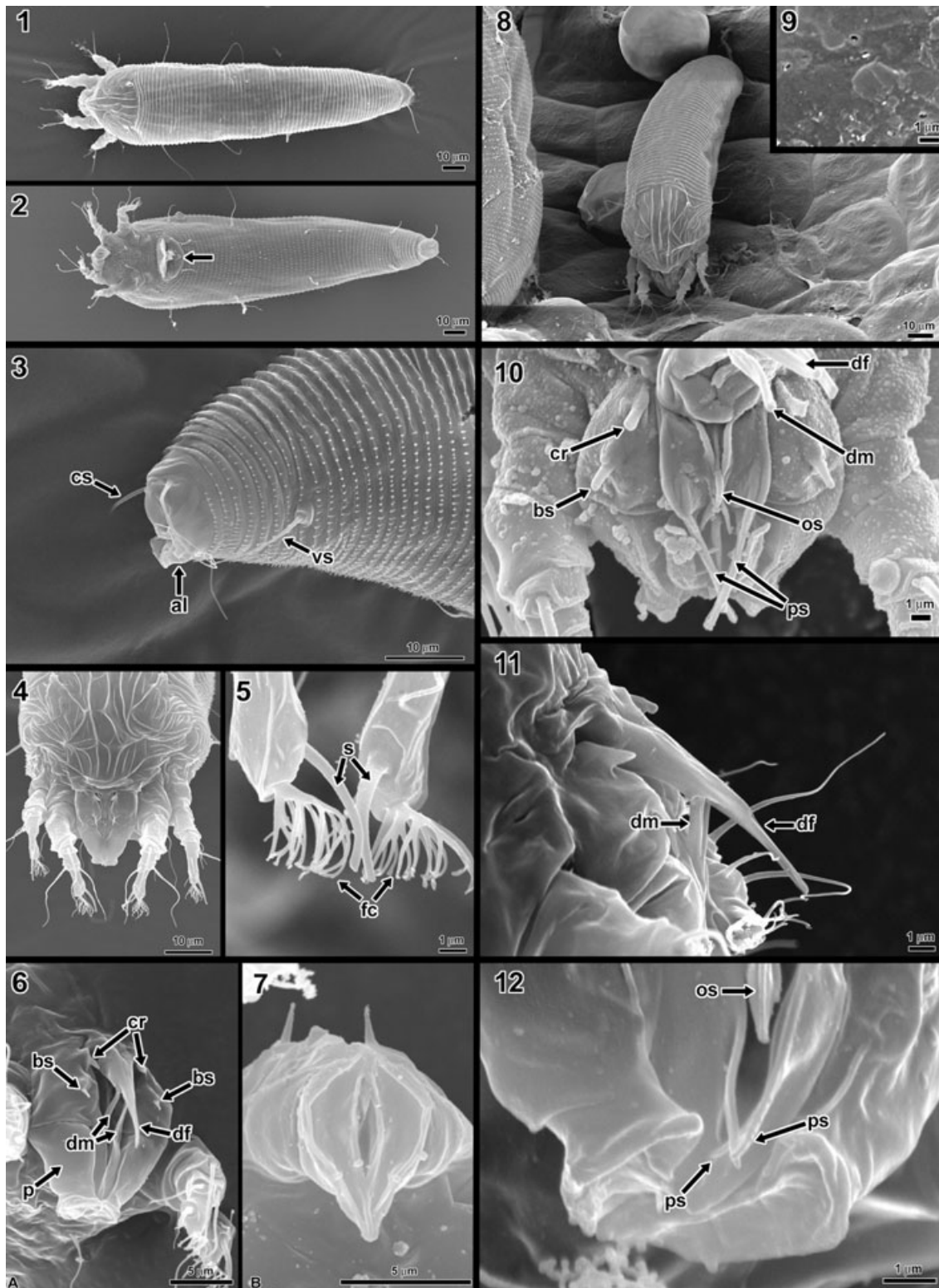
sectioned. For transmission electron microscopy, the tissue was stained *en bloc* with uranyl acetate, post-stained with lead citrate, and then examined on a JEOL 100CX microscope. For light microscopy, resin sections were cut (2–5 µm thick), stained with toluidine blue and examined using an Olympus BH-2 microscope.

## RESULTS

Mites removed from *L. microphyllum* subpinnae are shown in Figures 1,2. The general morphology is of the vermiform type, characterised by a complex gnathosoma, prodorsum, opisthosoma and two pairs of legs. The opisthosoma has three pairs of ventral setae and numerous rows of small annuli (Figs 1–3). Accessory setae are absent from the opisthosoma (Fig. 3). The ends of the legs have featherclaws and solenidia (Figs 4,5). Unguinal setae are absent from the tarsi. The genital region is on the ventral surface, just posterior to the legs (Fig. 2).

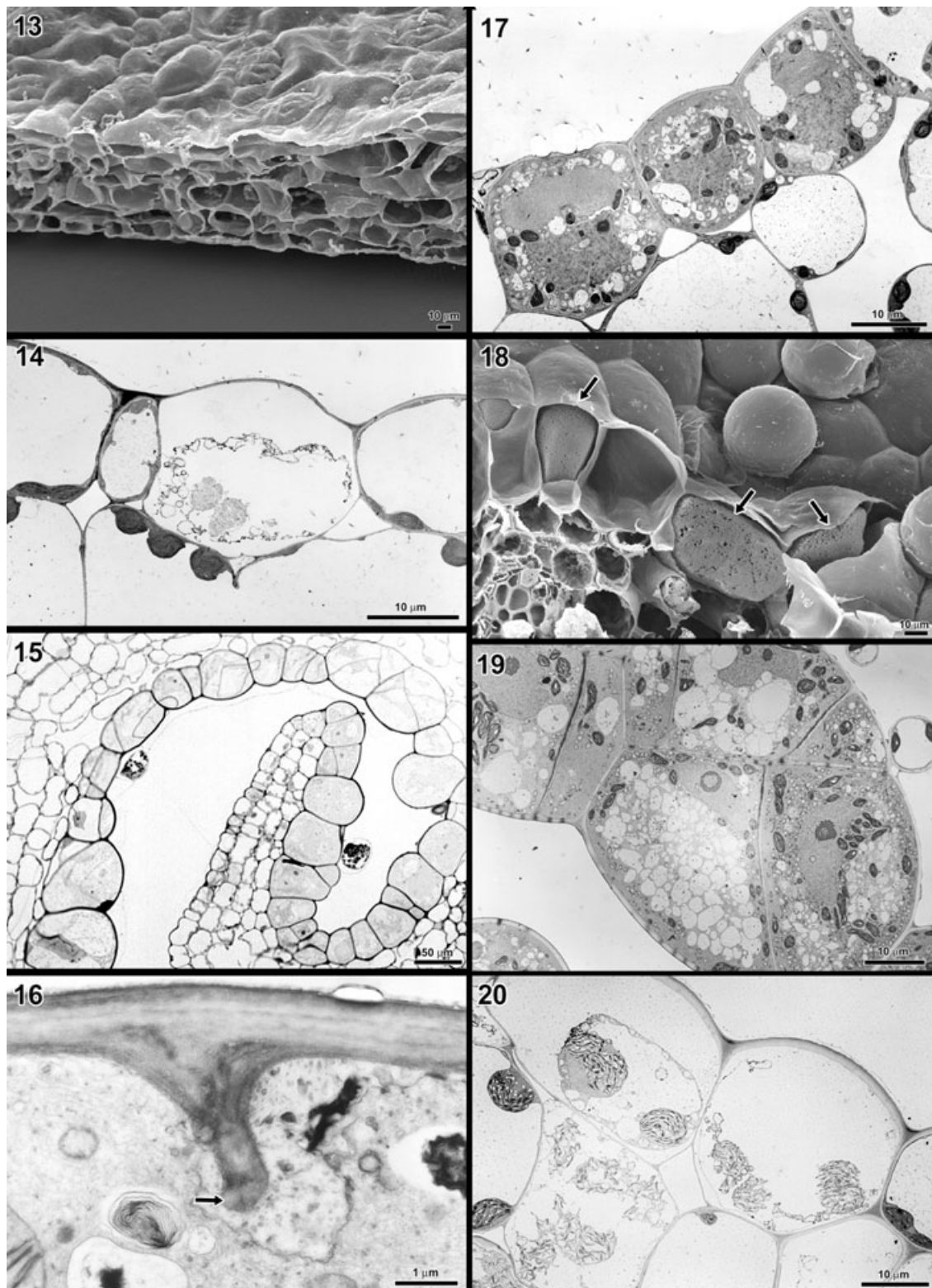
Mouthparts of these eriophyoid mites are complex and highly adapted for feeding on the host plants. The gnathosoma includes short pedipalps, a prominent subcapitulum (rostrum) and the stylets. During feeding, the pedipalps (Figs 6,7) form a suction type of attachment to the host plant while the anal sucker anchors the posterior end (Fig. 8). Damage to the fern epidermal wall, caused by stylet probes at feeding sites, was evident throughout the pinnae curls (Fig. 9). The stylets are visible only when the mite is feeding. There appear to be nine stylets in the stylet sheath of the mite. The most prominent stylets are the paired cheliceral stylets. These stylets taper towards their apex and each is divided into the digitus fixus, a fixed digit, and the digitus mobilis, a movable digit (Figs 6,10,11). The cheliceral stylets are the first to penetrate the host pinna epidermal cells. The pedipalpal stylets are shown in Figures 10,12. The distal end of one of the stylets appears to be branched, or there may be two pairs of pedipalpal stylets. The central unpaired stylet is the oral stylet (labrum) (Figs 10,12).

The mesophyll layer of the *L. microphyllum* subpinna is composed of relatively uniform parenchyma cells (Fig. 13) and scattered vascular bundles. The epidermal layers have few chloroplasts and limited cytoplasm (Fig. 14). Mite feeding on the subpinna epidermis induces rolling and, on some subpinnae the whole margin may be affected. Increased epidermal cell size and cell division are stimulated by mite feeding, and results in the leaf margin rolling over, either upward or downward, and inward (Fig. 15) on itself as many as two or three times. An epidermal cell-wall extension develops in response to *F. perrepae* feeding activity (Fig. 16). Mite feeding also stimulates the epidermal layer to become a nutritive tissue (Figs 17,18). The enlarged epidermal cells greatly increase their cytoplasmic contents as the subpinna continues to roll (Figs 17–19). Continued feeding by the adults and immatures leads to subpinna necrosis (Fig. 20) and premature defoliation of *L. microphyllum*, gradually debilitating the plant over time. The subpinna eventually dries and falls off the frond, before



**Figs 1–12.** *Floracarus perrepae*: (1) dorsal view; (2) ventral view of female with genital aperture (arrow); (3) posterior view of opisthosoma showing caudal seta (cs), third ventral seta (vs), and anal lobe (al); (4) dorsal view of anterior showing the gnathosoma and paired legs; (5) lateral view of tarsi I (empodial featherclaws (fc), solenidia (s)); (6) gnathosoma with cheliceral stylets (digitus fixus (df) and digitus mobilis (dm)) extended, and cheliceral retainers (cr), basal (palpcoxal) setae (bs), and pedipalp (p); (7) distal ends of palps forming 'suction pad'; (8) on the abaxial surface of a *Lygodium microphyllum* subpinna; (9) feeding sites on the epidermal surface of *L. microphyllum*; (10) complete set of stylets (digitus fixus (df), digitus mobilis (dm), oral stylet (os), pedipalpal stylets (ps), cheliceral retainer (cr), and basal seta (bs)); (11) lateral view of the cheliceral stylets (digitus fixus (df) and digitus mobilis (dm)); (12) oral stylet (os) and pedipalpal stylets (ps).





**Figs 13–20.** *Lygodium microphyllum*: (13) scanning electron micrograph of surface and sectional view of a non-curved area of a subpinna; (14) transmission electron micrograph of abaxial epidermis in a non-rolled area of the subpinna; (15) light micrograph of a subpinna curl showing enlarged nutritive abaxial epidermis; (16) transmission electron micrograph of an epidermal cell-wall extension (arrow) developed in response to *Floracarus perrepae* feeding activity; (17) transmission electron micrograph of the early stage of nutritive epidermal cell development within the subpinna leaf roll; (18) scanning electron micrograph of a surface and cross-sectional view of enlarged nutritive epidermal cells (arrows); (19) transmission electron micrograph of nutritive tissue in the subpinna curl; (20) transmission electron micrograph of necrotic epidermal and mesophyll tissue caused by continued *F. perrepae* feeding activity.

which time the adults move to another young subpinna to feed and initiate new curls. Eventually, the mite-induced leaf necrosis and death equals the level of new growth that appears.

We found that the *F. perrepae* genotype collected from south-eastern Queensland (Logan) was not able to induce leaf rolling in the invasive *L. microphyllum* genotype from Florida, but it readily induced leaf rolls in the local south-eastern Queensland genotype of the fern. Differences in the structure of the epidermal layer of *L. microphyllum* from the south-eastern Queensland and Florida genotypes were investigated to determine if these could account for the differences in acceptance by *F. perrepae*. We were unable to identify any specific morphological or anatomical differences between the ferns from different locations that might account for the different level of acceptance by the mite.

## DISCUSSION

Knihinicki and Boczek (2002) described this mite and mentioned that there are no unguinal and caudal setae; we confirmed this.

Lindquist (1996) and Nuzzaci and Alberti (1996) stated that Eriophyidae have seven stylets while some taxa, particularly Phytopidae and Diptilomiopidae, have nine stylets. *Floracarus perrepae* belongs to the family Eriophyidae. If there are indeed two pairs of pedipalpal stylets, the total number of stylets would be nine and might place this species beyond the limits of the family Eriophyidae. Additional ultrastructural studies will be required to resolve this issue.

The feeding mechanism of *F. perrepae* is poorly understood and further interpretation of the function of the various components of the gnathosoma is required. Feeding by *F. perrepae* on *L. microphyllum* stimulates the development of nutritive tissue causing the formation of tight leaf rolls on the subpinnae. The leaf rolls maintain an environment favourable for the mite. The substantial difference in vulnerability of the two genotypes of *L. microphyllum* to the feeding activity of *F. perrepae* does not appear to be related to mite stylet length or plant epidermal cell wall structure. An investigation of the biochemical basis for the adapted genotype will allow a greater understanding of the successful interaction of the mite and the fern. The ability of the mite to induce large leaf rolls and nutritive tissue is an important factor in choosing the most-appropriate genotype as a biological control agent. Our findings illustrate the complex and profound interactions of eriophyid mites and their hosts. As for *F. perrepae*, this interaction with the two genotypes of *L. microphyllum* emphasises its extremely narrow host specificity and potential to impact its host. This function of the biology of *F. perrepae* is the basis for its efficacy and safety as a biological control agent.

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